Experimenting with Marker-assisted Selection in Confection Sunflower Germplasm Enhancement

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Abstract

This preliminary report describes the application of target region amplification polymorphism (TRAP) markers to marker-assisted selection (MAS) in sunflower. TRAP is a fairly new marker technique that takes the advantage of the existing DNA sequence information to generate polymorphic markers near the gene of interest. The objective of this project was to identify maintainer lines from a segregating population. The TRAP marker, a 162-basepair fragment, used in the experiment was amplified by a fixed primer designed against a sunflower expressed sequence tag (EST) that shows homology to the Petunia fertility restorer gene. Two to nine seedlings from each of the 141 F_3 families were genotyped with the TRAP marker. Forty families were identified as potential maintainer lines and used as male parents in making test crosses with a confection male sterile line; the progeny were grown in the field to observe male fertility. Only 18 of the 40 selected F_3 families were of true *rf1*/*rf1* genotype and can be used as maintainer lines. Although the frequency of recovery of the desirable genotype was 45%, it was significantly higher than that expected from a random sample for a monogenic trait where the homozygous genotype frequency is 25%. This experiment suggested that MAS can substantially increase the chance of recovering individuals with the desirable genotype from a segregating population. Using a marker tightly linked to the target gene and genotyping enough individuals per family are essential to ensure a successful MAS experiment.

Introduction

Genetic markers associated with traits of economical importance can be used by plant breeders as selection tools to increase efficacy (Darvasi and Soller, 1994). Marker-assisted selection (MAS) has been practiced for major crop plants in both public and private breeding programs and numerous positive results have been published (Moreau et al., 2005; Steele et al., 2006). Sporadic reports of MAS in sunflower exist in the literature (Lawson et al., 1998; Brahm et al., 2000).

Oilseed sunflower has been the research focus of the Sunflower Research Unit, USDA-ARS Northern Crop Science Laboratory at Fargo, ND, and numerous oilseed sunflower germplasm lines with improved agronomic traits have been released. One of our breeding projects aimed to incorporate desirable traits such as disease resistance and herbicide resistance into confection sunflower lines for public release.

Commercial sunflower hybrids are produced by utilizing the cytoplasmic male sterility (CMS) system in which three lines are required. Lacking fertility restorer (*Rf*) gene(s), the male-sterile lines (A lines) are unable to produce functional pollen. The male-sterile character of the A lines is maintained by the maintainer lines (B lines) which also lack the *Rf* gene but possess a normal cytoplasm. The restorer lines (R lines) have genes for fertility restoration of the cytoplasmic male sterility and are used as pollinators for the A lines to produce uniform, high yielding hybrids. The only CMS cytoplasm used in sunflower is the PET1 CMS cytoplasm, which arose from an interspecific cross between a wild sunflower species, *Helianthus petiolaris* and the cultivated sunflower, *H. annuus* (Leclercq, 1969; Serieys, 1996). It has been demonstrated that two dominant nuclear genes, Rf_1 and Rf_2, are necessary to restore the male fertility, but Rf_2 is present in nearly all sunflower lines. Thus, in practice, breeding for R lines only needs to introduce the Rf_1 gene and does not require a progeny test to check the presence of the introduced Rf_1 gene if the selected lines have the PET1 CMS cytoplasm. As a result, all or most all of the R lines possess the PET1 cytoplasm. On the other hand, breeding for B lines requires a progeny test of the cross between a selected line with a male-sterile line to determine whether the selected line has the rf_1 gene to maintain the male sterility. Therefore, a DNA marker associated with the Rf_1 gene will be useful in selecting for maintainer and restorer lines with normal cytoplasm. In this paper, we report an experiment on the application of a target region amplification polymorphism (TRAP) marker to MAS in breeding for confection sunflower B lines.

Materials and Methods

Twenty-four USDA sunflower lines (12 R lines and 12 B lines) that were released to the public by USDA-ARS and the North Dakota State University Experiment Station during the period of 1973 to 2005 were used in the initial screening for marker-trait association to identify markers associated with *Rf_1* gene. The MAS experiment was carried out with seedlings of 141 F_3 families of three different expected genotypes at the *Rf_1* locus, namely, *Rf_1/Rf_1*, *Rf_1/rf_1* and *rf_1/rf_1*. The pedigree of the segregating population is shown in Figure 1.

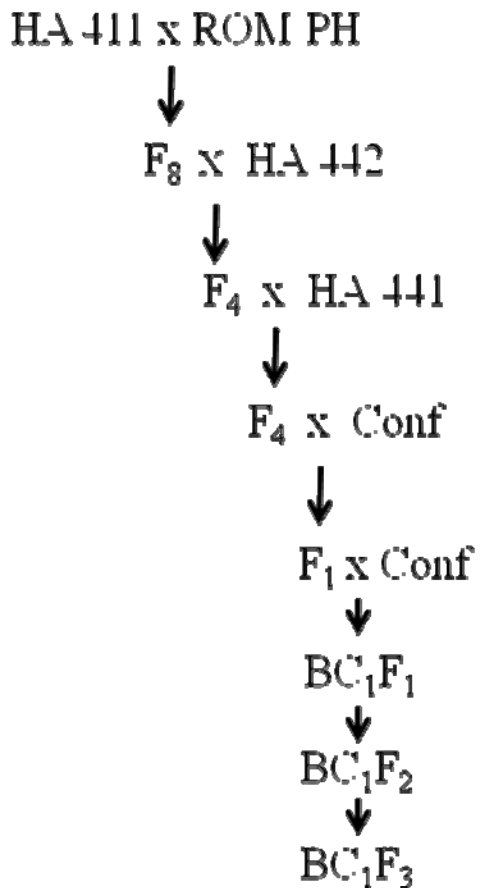
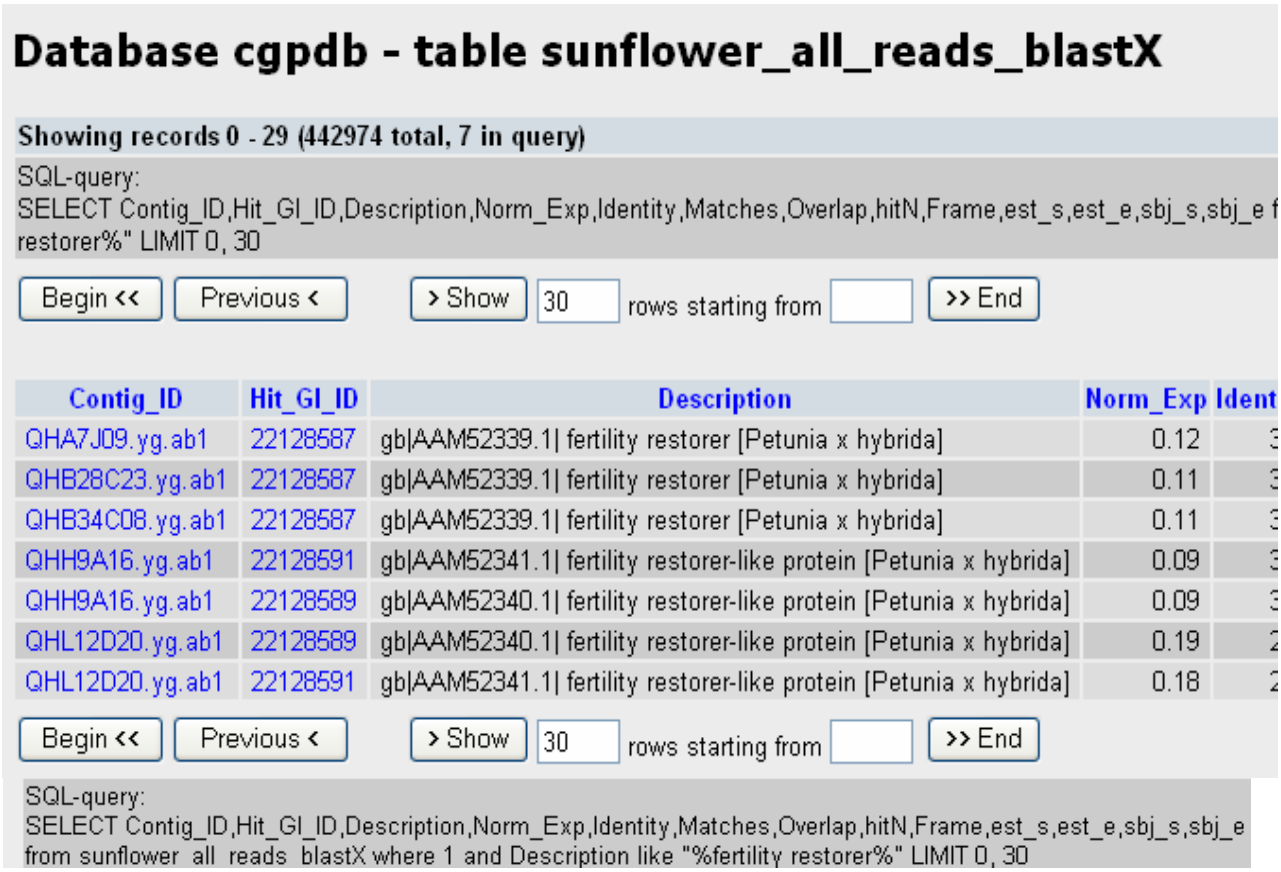


Figure 1. Pedigree of the segregating BC_1 F_3 population used in the current experiment. Three USDA-ARS released B lines (HA 411, HA 442 and HA 441) and two breeding populations (ROM PH and CONF) were used in the breeding project. Both HA 411 and HA 441 are Sclerotinia tolerant and HA 442 is imidazolinone resistant. ROM PH is a Phomopsis stem canker resistant population obtained from Romania and CONF is a bulk pollen from two confection sunflower plant introductions from China, Nei Mongo 1 (Ames 21671) and JB 4 (Ames 10101).

Genomic DNA was prepared from two-week-old seedlings with a DNeasy 96 Plant Kit (Qiagen, Valencia, CA*) following the manufacturer's instructions. The published TRAP procedures (Hu and Vick, 2003) and the updated TRAP protocol (Hu, 2006) were followed for TRAP marker development and application. Seventeen fixed primers were designed against the DNA

sequences coding for the fertility restorer-like pentatricopeptide (PPR) repeat-containing protein (Figure 2). Fifteen of these primers were against the five sunflower expressed sequence tags (EST) identified from the Compositae Genome Project Database (http://cgpdb.ucdavis.edu/) and the other two were against the petunia *Rf* gene, the first cloned CMS *Rf* gene from higher plants (Bentolila et al., 2002).



Database cgpdb - table sunflower_all_reads_blastX

Showing records 0 - 29 (442974 total, 7 in query)

SQL-query:
SELECT Contig_ID,Hit_GI_ID,Description,Norm_Exp,Identity,Matches,Overlap,hitN,Frame,est_s,est_e,sbj_s,sbj_e f
restorer%" LIMIT 0, 30

Begin << | Previous < | > Show 30 rows starting from | >> End

Contig_ID	Hit_GI_ID	Description	Norm_Exp	Ident
QHA7J09.yg.ab1	22128587	gb|AAM52339.1| fertility restorer [Petunia x hybrida]	0.12	3
QHB28C23.yg.ab1	22128587	gb|AAM52339.1| fertility restorer [Petunia x hybrida]	0.11	3
QHB34C08.yg.ab1	22128587	gb|AAM52339.1| fertility restorer [Petunia x hybrida]	0.11	3
QHH9A16.yg.ab1	22128591	gb|AAM52341.1| fertility restorer-like protein [Petunia x hybrida]	0.09	3
QHH9A16.yg.ab1	22128589	gb|AAM52340.1| fertility restorer-like protein [Petunia x hybrida]	0.09	3
QHL12D20.yg.ab1	22128589	gb|AAM52340.1| fertility restorer-like protein [Petunia x hybrida]	0.19	2
QHL12D20.yg.ab1	22128591	gb|AAM52341.1| fertility restorer-like protein [Petunia x hybrida]	0.18	2

Begin << | Previous < | > Show 30 rows starting from | >> End

SQL-query:
SELECT Contig_ID,Hit_GI_ID,Description,Norm_Exp,Identity,Matches,Overlap,hitN,Frame,est_s,est_e,sbj_s,sbj_e
from sunflower_all_reads_blastX where 1 and Description like "%fertility restorer%" LIMIT 0, 30

Figure 2. Result of searching fertility restorer homolog ESTs from the Compositae Genome Project Database (http://cgpdb.ucdavis.edu/, accessed in January 2006). Five sunflower ESTs showed homology to the petunia fertility restorer protein gene.

In the initial screening, these fixed primers were used in combination with the infrared dye labeled arbitrary primers to amplify the B and R DNA pools, which contained equal amounts of DNA isolated from each of the 12 B lines and 12 R lines, respectively. Primer combinations that amplified polymorphic fragments between the two pools were used to amplify DNA from individual R and B lines.

Results and discussion

1. Identification and confirmation of the TRAP marker associated with *Rf_1* gene

One marker from the initial screening was a fragment of 162 basepairs amplified by a primer combination of QHL12D20a and Trap13-800 from the R line DNA pool. When testing with DNA from 24 individual lines this fragment showed up in 11 of the R lines and in only one of the 12 B lines (Figure 3). The association between this fragment with the *Rf_1* gene was further confirmed by amplifying from DNA samples of three segregating F_3 families (16 seedlings each) of known genotypes at the *Rf_1* locus (*Rf_1/Rf_1*, *Rf_1/rf_1* and *rf_1/rf_1*). As expected, this fragment was present in all 16 seedlings from the *Rf_1/Rf_1* family, absent in the *rf_1/rf_1*family and segregating in the *Rf_1/rf_1* family. This marker was then used in the MAS experiment.

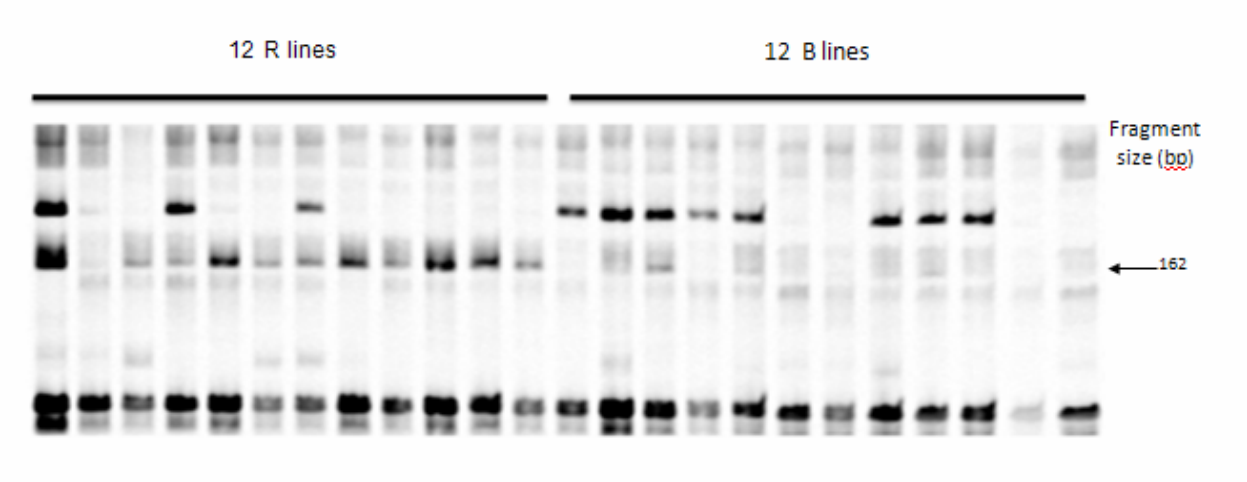


Figure 3. The TRAP marker, a 162-bp fragment (arrow), was present in 11 of the 12 R lines and absent in all but one of the 12 B lines.

2. Genotyping the F_3 families and progeny test of the selected families

Due to constraints of resources, we had to limit the number of seedlings per family to be genotyped. Eight seeds from each of the 141 F_3 families were planted in the greenhouse and resulted in a total of 698 seedlings (one to nine seedlings per family) for genotyping. The primer combination of QHL12D20a and Trap13-800 revealed that there were 46 homozygous *Rf_1/Rf_1*, 55 heterozygous *Rf_1/rf_1* and 40 homozygous *rf_1/rf_1* families. This observed ratio deviated from the expected 1:2:1 segregating ratio ($x^2 = 7.33$, $P<0.05$). The excess of both homozygous

genotypes might have resulted from too few individuals per family used in genotyping. Forty families lacking the 162-bp fragment were identified as the *rf1*/*rf1* genotype. The seedlings from these 40 families were grown to maturity and used as male parents in making test crosses with a confection A line. The progeny of these 40 test crosses were grown in the field in Fargo during the 2006 growing season to observe male fertility.

Field observation on the fertility of the 40 test crosses (20 to 25 plants per cross) recorded six homozygous Rf_1/Rf_1, 16 heterozygous Rf_1/rf_1 and 18 homozygous rf_1/rf_1 families. This observed ratio again deviated from the expected 1:2:1 segregating ratio ($x^2 = 8.8$, $P<0.025$), indicating that MAS increased substantially the number of the targeted genotype. The 45% success rate (18 out of 40 selected F_3 families were of true *rf1*/*rf1* genotype) seemed low for a MAS experiment on a monogenic trait. However, if 40 families were randomly chosen to make test crosses, only 10 homozygous rf_1/rf_1 families would be expected. MAS in this experiment almost doubled the number of families with the desirable genotype. The low success rate could be explained by two factors: 1) the small number of seedlings per family used, as discussed earlier, and 2) the marker used is not tightly linked to the targeted gene Rf_1, and that recombination between the marker and the Rf_1 gene decreased the selection efficiency.

In conclusion, MAS is likely to accelerate breeding progress by increasing substantially the chance of recovering individuals with the desirable genotype from a segregating population. Using a marker tightly linked to the target gene and genotyping enough individuals per family are essential to ensure a successful MAS experiment.

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*Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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